Supplemental Materials

CssA   ## CssB

(A)
Linear model

(B)
Surface
accessibility
model

(C)
Hydrophobicity
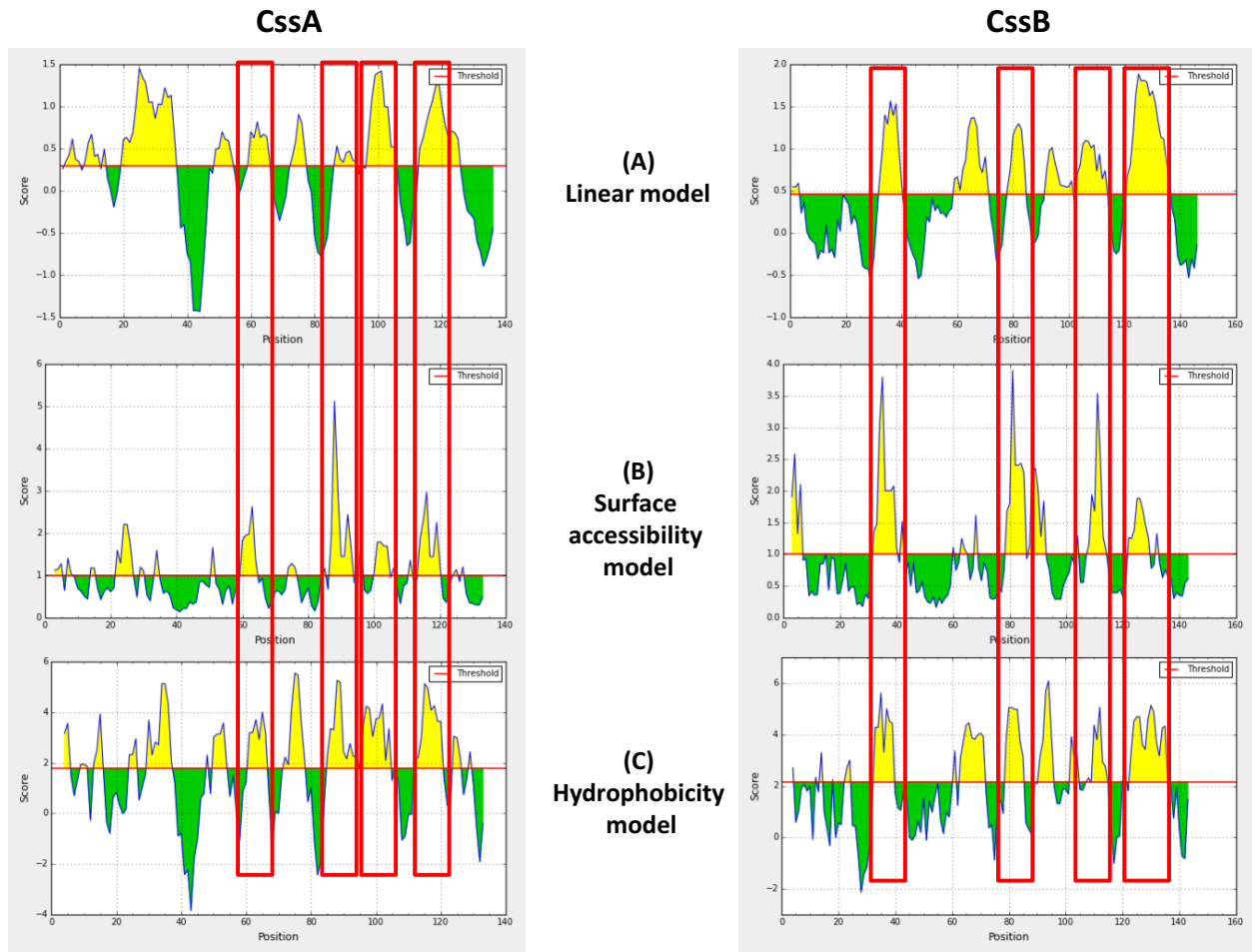model

Figure S1. *In silico* **evaluation of CssA and CssB B cell epitopes.** The mature amino acid sequence of CssA and CssB subunits (GenBank Accession Numbers AAB51361 and AAB51362, respectively) from the ETEC E8775 CS6 colonization factor were analyzed by three open access B cell epitope prediction software (http://tools.iedb.org/bcell/). (A) Linear Model; (B) Surface Accessibility Model; (C) Hydrophobicity Model. For all models, default threshold was used. Red boxes indicate areas of the proteins that were identified as possible B cell epitopes by all approaches.

Table S1. Construction of expression clones for CS6 dimeric and trimeric fusions originating from CssA.

Clone	Primer Set Description	Primer - Sequence
pET24 - cssA	PCR of *cssA* from ETEC E8775 with internal <u>SnaBI</u> – digested with NdeI and XhoI and cloned into *pET-24a(+)*	AF - GCGGCGGCG<u>CATATG</u>AGAACAGAAATAGCGACTAAAAAC
		AR - GCGGCG<u>CTCGAG</u>TTGTTTATTGTCGTT<u>TACGTA</u>GTAACCAACCATAACC
pET24 – dscₐcssA 6xhis	Oligos containing DNKQ linker and dsc16 from *cssA* – annealed and digested with <u>SnaBI</u> and <u>XhoI</u>, then ligated to *pET24 - cssA* at <u>SnaBI</u> and <u>XhoI</u>	dsc16AF - GCGGCGGCG<u>TACGTA</u>AACGACAATAAACAACGTACCGAGATTGCCACCAAGAATTTTCCGGTGAGCACCACCATCAGC<u>CTCGAG</u>GCGGCG
		dsc16AR - CGCCGC<u>CTCGAG</u>GCTGATGGTGGTGCTCACCGGAAAATTCTTGGTGGCAATCTCGGTACGTTGTTTATTGTCGTT<u>TACGTA</u>CGCCGCCGC
pET24-dscₐcssAA 6xhis	PCR of *cssA* from *pET24 - dscₐcssA 6xhis* – digested with <u>ZraI</u> and <u>XhoI</u> then ligated to *pET24-dscₐcssA 6xhis* at <u>SnaBI</u> and <u>XhoI</u>	A1F - GGCGGCGGC<u>GACGTC</u>AACGACAATAAACAAAGAACAGAAATAGCGACTAAAAACTTCCC
		dscAR - GCGGCG<u>CTCGAG</u>GCTGATGGTGGTGC
pET24-dscᵦcssAB 6xhis	PCR of *cssB* from *pET24 - dscᵦcssB 6xhis* – digested with <u>ZraI</u> and <u>XhoI</u> then ligated to *pET24-dscₐcssA 6xhis* at <u>SnaBI</u> and <u>XhoI</u>	B1F- GGCGGCGGC<u>GACGTC</u>AACGACAATAAACAAGGAAACTGGCAATATAAATCTCTGG
		dscBR - GCGGCG<u>CTCGAG</u>CTGTTCGATGTTCACG
pET24-dscₐcssAB 6xhis	Oligos containing DNKQ linker and dsc16 from *cssA* – annealed and digested with <u>Cac8I</u> and <u>XhoI</u> then ligated to *pET24-cssAB* at <u>SnaBI</u> and <u>XhoI</u>	dsc16A1F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAAGAACAGAAATAGCGACTAAAAACTTCCCAGTATCAACGACTATTTCA<u>CTCGAG</u>GCGGCG
		dsc16A1R –

		CGCCGC<u>CTCGAG</u>TGAAATAGTCGTTGATACTGGGAAGTT TTTAGTCGCTATTTCTGTTCTTTGTTTATTGTCATT<u>GCTA GC</u>CGCCGCCGC
pET24-ntd_dsc_AcssAB 6xhis	Site directed mutagenesis performed to delete 15 N-terminal amino acids of *cssA* in *pET24-dsc_AcssAB 6xhis*	ntdABF - GAAGGAGATATACATATGTCAAAAAGTTTTTTTGCACCT G
		ntdABR - CAGGTGCAAAAAAACTTTTTGACATATGTATATCTCCTT C
pET24-ntd_dsc_BcssAB 6xhis	Site directed mutagenesis performed to delete 15 N-terminal amino acids of *cssA* in *pET24-dsc_BcssAB 6xhis*	ntdABF - GAAGGAGATATACATATGTCAAAAAGTTTTTTTGCACCT G
		ntdABR - CAGGTGCAAAAAAACTTTTTGACATATGTATATCTCCTT C
pET24-dsc_AcssABA 6xhis	PCR of *cssBA* from *pET24-dsc_AcssBA 6xhis* – digested with <u>ZraI</u> and <u>XhoI</u> then ligated to *pET24 – cssA* at <u>SnaBI</u> and <u>XhoI</u>	B1F - GGCGGCGGC<u>GACGTC</u>AACGACAATAAACAAGGAAACTG GCAATATAAATCTCTGG
		dscAR - GCGGCG<u>CTCGAG</u>GCTGATGGTGGTGC
pET24-cssAB	PCR of *cssB* from *pET24-cssB* – digested with <u>ZraI</u> and <u>XhoI</u> then ligated to pET24-cssA at <u>SnaBI</u> and <u>XhoI</u>	B1F - GGCGGCGGC<u>GACGTC</u>AACGACAATAAACAAGGAAACTG GCAATATAAATCTCTGG
		BR - GCGGCG<u>CTCGAG</u>TTGTTTATTGTCATT<u>TACGTA</u>AAAATGA TACAGTCAAATTTCC
pET24-dsc_BcssABB 6xhis	PCR of *cssB* from *pET24-dsc_BcssB 6xhis* – digested with <u>Cac8I</u> and <u>XhoI</u> then ligated to *pET24-cssAB* at <u>SnaBI</u> and <u>XhoI</u>	B2F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAGGAAACTG GCAATATAAATCTCTGG
		dscBR - GCGGCG<u>CTCGAG</u>CTGTTCGATGTTCACG

Table S2. Construction of expression clones for CS6 dimeric and trimeric fusions originating from CssB.

Clone	Primer Set Description	Primer - Sequence
pET24-cssB	PCR of *cssB* from ETEC E8775 – digested with NdeI and XhoI and cloned into *pET-24a(+)*	BF - GCGGCGGCG<u>CATATG</u>GGAAACTGGCAATATAAATCTCTG
		BR - GCGGCG<u>CTCGAG</u>TTGTTTATTGTCATT<u>TACGTA</u>AAAATGATACAGTCAAATTTCC
pET24-dsc_BcssB 6xhis	Oligos containing DNKQ linker and dsc16 from *cssB* –digested with Cac8I and XhoI then ligated to pET24-cssB at SnaBI and XhoI	dsc16BF - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAGGCAATTGGCAGTACAAGAGCCTCGACGTGAACGTGAACATCGAACAG<u>CTCGAG</u>GCGGCG
		dsc16BR - CGCCGC<u>CTCGAG</u>CTGTTCGATGTTCACGTTCACGTCGAGGCTCTTGTACTGCCAATTGCCTTGTTTATTGTCATT<u>GCTAGC</u>CGCCGCCGC
pET24-dsc_BcssB 6xhis SnaBI	Site-directed mutagenesis to incorporate SnaBI before the DNKQ linker for the addition of *cssB*	sdm BF - GGAAATTTGACTGTATCATTT<u>TACGTA</u>AATGACAATAAACAAGGCAATTGG
		sdm BR - CCAATTGCCTTGTTTATTGTCATT<u>TACGTA</u>AAAATGATACAGTCAAATTCC
pET24-dsc_AcssBA 6xhis	PCR of *cssA* from *pET24 - dsc_AcssA 6xhis* - digested with Cac8I and XhoI then ligated to *pET24-dsc_BcssB 6xhis SnaBI* at SnaBI and XhoI	A2F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAAGAACAGAAATAGCGACTAAAAACTTCCC
		dscAR - GCGGCG<u>CTCGAG</u>GCTGATGGTGGTGC
pET24-dsc_BcssBB 6xhis	PCR of *cssB* from *pET24 - dsc_BcssB 6xhis* – digested with Cac8I and XhoI then ligated to *pET24-dsc16cssB 6xhis SnaBI* at SnaBI and XhoI	B2F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAGGAAACTGGCAATATAAATCTCTGG
		dscBR - GCGGCG<u>CTCGAG</u>CTGTTCGATGTTCACG
pET24-dsc_BcssBA 6xhis		dsc16B1F - GCGGCGGCG<u>TACGTA</u>AACGACAATAAACAAGGAAACTGGCAAT

	Oligos containing DNKQ linker and dsc16 from *cssB* – digested with <u>SnaBI</u> and <u>XhoI</u> then ligated to *pET24-dsc_ACssBA 6xhis* at <u>SnaBI</u> and <u>XhoI</u>	ATAAATCTCTGGATGTAAATGTAAATATTGAGCAA<u>CTCGAG</u>CGCCGC
		dsc16B1R - GCGGCG<u>CTCGAG</u>TTGCTCAATATTTACATTTACATCCAGAGATTTATATTGCCAGTTTCCTTGTTTATTGTCGTT<u>TACGTA</u>CGCCGCCGC
pET24-ntd_dsc_ACssBA 6xhis	Site directed mutagenesis performed to delete 14 N-terminal amino acids of *cssB* in *pET24-dsc_ACssBA 6xhis*	ntdBAF - GAAGGAGATATACATATGGAGCAAAATTTTATTCCAG
		ntdBAR - CTGGAATAAAATTTTGCTCCATATGTATATCTCCTTC
pET24-ntd_dsc_BCssBA 6xhis	Site directed mutagenesis performed to delete 14 N-terminal amino acids of *cssB* in *pET24-dsc_BCssBA 6xhis*	ntdBAF - GAAGGAGATATACATATGGAGCAAAATTTTATTCCAG
		ntdBAR - CTGGAATAAAATTTTGCTCCATATGTATATCTCCTTC
pET24-dsc_BCssBAB 6xhis	PCR of *cssAB* from *pET24-dsc_BCssAB 6xhis* – digested with <u>Cac8I</u> and <u>XhoI</u> then ligated to *pET24-cssB* at <u>SnaBI</u> and <u>XhoI</u>	A2F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAAGAACAGAAATAGCGACTAAAAACTTCCC
		dscBR - GCGGCG<u>CTCGAG</u>CTGTTCGATGTTCACG
pET24-dsc_ACssBAA 6xhis	PCR of *cssA* from *pET24 - dsc_ACssA 6xhis* – digested with <u>ZraI</u> and <u>XhoI</u> then ligated to *pET24-dsc_ACssBA 6xhis* at <u>SnaBI</u> and <u>XhoI</u>	A1F - GGCGGCGGC<u>GACGTC</u>AACGACAATAAACAAAGAACAGAAATAGCGACTAAAAACTTCCC
		dscAR - GCGGCG<u>CTCGAG</u>GCTGATGGTGGTGC
pET24-dsc_ACssBBA 6xhis	PCR of *cssBA* from *pET24- dsc_ACssBA 6xhis* - digested with <u>Cac8I</u> and <u>XhoI</u> then ligated to *pET24-cssB* at <u>SnaBI</u> and <u>XhoI</u>	B2F - GCGGCGGCG<u>GCTAGC</u>AATGACAATAAACAAGGAAACTGGCAATATAAATCTCTGG
		dscAR - GCGGCG<u>CTCGAG</u>GCTGATGGTGGTGC